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Cerebral differences between dopamine-resistant and dopamine-responsive Parkinson's tremor

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Rest tremor in Parkinson's disease is related to cerebral activity in both the basal ganglia and a cerebello-thalamo-cortical circuit. Clinically, there is strong interindividual variation in the therapeutic response of tremor to dopaminergic medication. This observation casts doubt on the idea that Parkinson's tremor has a dopaminergic basis. An interesting alternative explanation is that interindividual differences in the pathophysiology of tremor may underlie this clinical heterogeneity. Previous work showed that dopaminergic medication reduces Parkinson's tremor by inhibiting tremulous activity in the pallidum and thalamus, and this may explain why some tremors are dopamine-responsive. Here we test the hypothesis that dopamine-resistant resting tremor may be explained by increased contributions of non-dopaminergic brain regions, such as the cerebellum. To test this hypothesis, we first performed a levodopa challenge test in 83 tremulous Parkinson's disease patients, and selected 20 patients with a markedly dopamine-responsive tremor (71% reduction) and 14 patients with a markedly dopamine-resistant tremor (6% reduction). The dopamine response of other core motor symptoms was matched between groups. Next, in all 34 patients, we used combined EMG-functional MRI to quantify tremor-related brain activity during two separate sessions (crossover, double-blind, counterbalanced design): after placebo, or after 200/50 mg dispersible levodopa/benserazide. We compared tremor-related brain activity between groups and medication sessions. Both groups showed tremor amplitude-related brain activity in a cerebello-thalamo-cortical circuit. Dopamine-resistant tremor patients showed increased tremor-related activity in non-dopaminergic areas (cerebellum), whereas the dopamine-responsive group showed increased tremor-related activity in the thalamus and secondary somatosensory cortex (across medication sessions). Levodopa inhibited tremor-related thalamic responses in both groups, but this effect was significantly greater in dopamine-responsive patients. These results suggest that dopamine-resistant tremor may be explained by increased cerebellar and reduced somatosensory influences onto the cerebellar thalamus, making this region less susceptible to the inhibitory effects of dopamine.

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Abbreviations: MDS-UPDRS = Movement Disorders Society-Unified Parkinson's Disease Rating Scale; VLpv = ventrolateral posterior nucleus, pars ventralis

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Introduction

Parkinson's disease is a progressive neurodegenerative disorder characterized among others by nigrostriatal dopamine depletion (Kish *et al.*, 1988). However, while dopaminergic medication effectively treats bradykinesia and rigidity, the effect on resting tremor is unpredictable and varies greatly between patients (Koller, 1986; Koller and Hubble, 1990). The cerebral mechanisms underlying this variable treatment response are unknown, limiting the development of alternative treatment strategies for dopamine-resistant tremor. In previous work we identified a cerebral tremor network in Parkinson's disease consisting of both the basal ganglia and a cerebello-thalamo-cortical motor circuit (Helmich *et al.*, 2011; Dirkx *et al.*, 2016). According to the 'dimmer-switch hypothesis', the basal ganglia initiate tremor-related activity, which is then propagated to the cerebello-thalamo-cortical circuit via the motor cortex (Dirkx *et al.*, 2016). In turn, the cerebello-thalamo-cortical circuit maintains and amplifies tremulous activity, which may occur via afferent signals that stabilize intrinsic tremor oscillations (Volkman *et al.*, 1996; Helmich, 2018).

Recent observations provide a number of possibilities that may explain between-patient variations in the clinical dopamine response of resting tremor. First, we recently found that a dopaminergic intervention influences tremor-related activity in the basal ganglia (pallidum) and the cerebellar receiving nucleus of the thalamus (ventrolateral posterior nucleus, pars ventralis, VLpv) (Dirkx *et al.*, 2017). Clinical dopamine responsiveness correlated with dopamine-induced inhibition of tremor activity in the VLpv, suggesting that dopamine-resistant tremor may be due to failure of dopamine to inhibit the VLpv. Second, patients with Parkinson's disease have extensive Lewy body pathology in the cerebellum, with links to the occurrence of (dopamine-resistant) tremor (Seidel *et al.*, 2017). Furthermore, it is clear from other (non-dopaminergic) tremor diseases, for example essential tremor (Muthuraman *et al.*, 2018), that cerebellar pathology can drive tremulous activity within the cerebello-thalamo-cortical circuit (Hopfner and Helmich, 2018). This suggests that dopamine-resistant tremor may be due to increased tremor-related activity in non-dopaminergic brain regions, particularly the cerebellum.

To test these possibilities, we carefully selected Parkinson's disease patients with either a dopamine-resistant or a dopamine-responsive tremor from a large cohort ($n = 83$) that underwent a clinical levodopa challenge, prior to functional MRI. Subsequently, we performed concurrent functional MRI and electromyography (EMG) on two separate sessions: OFF dopaminergic medication (placebo) and ON (levodopa) dopaminergic medication (standardized challenge of 200/50 mg levodopa/benserazide). We used these data to compare tremor-related brain activity between Parkinson's disease patients with a dopamine-resistant versus a dopamine-responsive tremor.

Materials and methods

Study population and design

We included 83 patients diagnosed with idiopathic Parkinson's disease (according to the UK Brain Bank criteria), with resting tremor of at least one arm, and a history of resting tremor. Exclusion criteria were: (i) neurological co-morbidity; (ii) signs of psychogenic tremor (e.g. entrainment or distractibility); (iii) known allergy against levodopa-benserazide or domperidone; and (iv) significant cognitive impairment [Mini-Mental State Examination (MMSE) (Folstein *et al.*, 1975) score < 24 or frontal assessment battery (FAB) (Dubois *et al.*, 2000) < 12]. The study was approved by the local ethics committee and written informed consent was collected before inclusion.

In all patients, we carried out a levodopa challenge test to select those patients showing a clear dopamine-responsive or dopamine-resistant tremor (Fig. 1A). Patients came to our clinic in the morning after overnight fasting, in a practically defined OFF state (i.e. > 12 h after their last dose of levodopa, > 30 h after their last dose of dopaminergic agonists and > 24 h after their last dose of beta-blockers) (Albanese *et al.*, 2001; Zach *et al.*, 2017) and after abstention from caffeine (tea, coffee) for at least 12 h. None of the included patients used beta-blockers to treat tremor; they were prescribed for hypertension or cardiac disease. Using clinical rating scales (Movement Disorders Society-Unified Parkinson's Disease Rating Scale; MDS-UPDRS) and accelerometry, patients were measured both before and after levodopa. For the ON state (levodopa) assessment, patients received a standard dose of 200/50 mg dispersible levodopa-benserazide (this was on average 75% higher than the patients' own morning dose) 1 h after 20 mg domperidone. There is currently no consensus on the definition of tremor dopamine responsiveness. This is why we used (arbitrary) predefined criteria, which we validated after data collection using an automated, data-driven clustering approach (supplementary analyses). We predefined dopamine-resistant tremor as a clinical improvement of $\leq 20\%$ on the MDS-UPDRS rest tremor score (item 17), and dopamine-responsive tremor as a clinical improvement of $\geq 60\%$ (Albanese *et al.*, 2001). Furthermore, only patients who showed $\geq 20\%$ improvement of rigidity and bradykinesia (determined using items 3–8 of the MDS-UPDRS) were included (Albanese *et al.*, 2001), to rule out trivial explanations for levodopa resistance, such as gastro-intestinal malabsorption (Nonnekes *et al.*, 2016). These criteria were registered at the Dutch Trial Registry prior to onset of the study (www.trialregister.nl; trial number NTR5042), although we later had to change the criterion for dopamine-responsive tremor from $\geq 60\%$ to $\geq 50\%$ to reach our target of 20 dopamine-responsive patients. All measurements took place from July 2014 until February 2016.

After inclusion, we compared limb rigidity (MDS-UPDRS item 3), limb bradykinesia (MDS-UPDRS items 4–8) and limb resting tremor (MDS-UPDRS item 17) between both groups using a 2×2 ANOVA with factors Group (dopamine-resistant versus responsive) and Medication (placebo versus dopamine) with *post hoc* one and two sample *t*-tests in SPSS (two-tailed) (Fig. 1 and Table 1). To objectively quantify tremor, we used a bi-axial piezoelectric accelerometer (Medifactory International; sampling frequency 128 Hz)

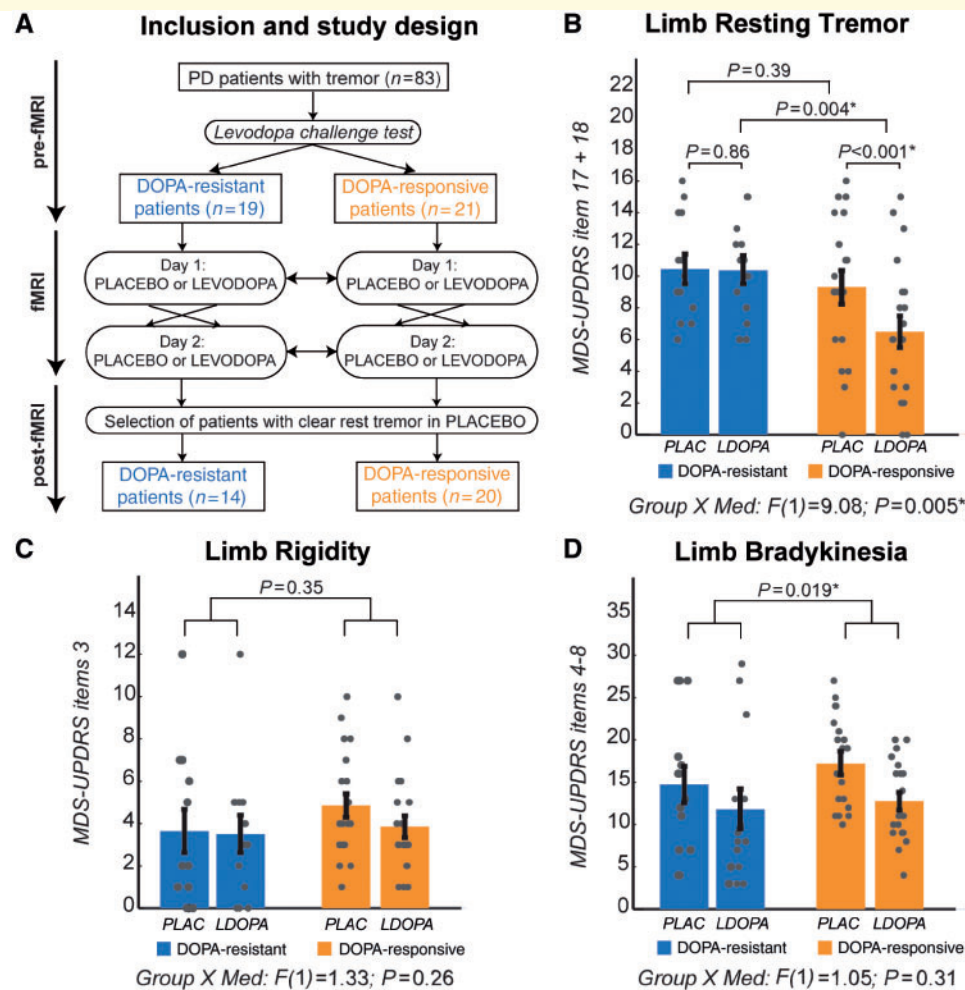


Figure 1 Study design and clinical results. (A) Study design and selection of patients. (B–D) Comparison of clinical dopaminergic response in each of the three cardinal symptoms in each group. It can be seen that only limb resting tremor improved following levodopa in the dopamine-responsive group (orange bars) but not in the dopamine-resistant group (blue bars). The bar graphs show the mean (\pm SEM) and all individual datapoints. There were no outliers (defined as lower/greater than mean \pm three times the standard deviation). Note that there was one dopamine-responsive patient who did not have a tremor during clinical evaluation in the placebo session, but who showed a clear peak in the power spectrum (frequency range) of the EMG/accelerometry signal during scanning, indicating that this patient did have a tremor during the functional MRI (fMRI) measurement. PD = Parkinson's disease.

attached to the affected upper extremity during the levodopa challenge test. Using FieldTrip (Oostenveld *et al.*, 2011), we calculated the mean tremor power values (log transformed) across three 1-min trials both at rest and during cognitive stress (i.e. counting backwards from 100 in steps of 7 as fast as possible), and during both OFF and ON (for details see Zach *et al.*, 2017) (Table 1).

Patients with a dopamine-resistant ($n=19$) or dopamine-responsive tremor ($n=21$) were scanned using functional MRI on two separate days (Fig. 1A). Patients came to our clinic in a practically defined OFF state. They received 20 mg of domperidone and either 200/50 mg dispersible levodopa-benserazide or a placebo (dispersible cellulose) in a crossover, counterbalanced design. There were no significant time differences between intake of substance and scanning in both groups and sessions [dopamine-resistant: placebo 52 ± 6 min, levodopa 53 ± 19 min; dopamine responsive: placebo 48 ± 10 min,

levodopa 48 ± 9 min (mean \pm standard deviation); Medication \times Group: $F(1,32)=0.053$, $P=0.82$]. Before and after scanning patients were evaluated clinically using the MDS-UPDRS by an experienced movement disorders specialist who was blinded to group assignment and the intervention. After scanning, we only included patients who had a clear resting tremor in (at least) the placebo state based on the EMG power spectrum measured during actual scanning. This resulted in 20 patients with a dopamine-responsive tremor and 14 patients with a dopamine-resistant tremor.

Image acquisition and preprocessing

Functional MRI was carried out on a 3 T MRI system (Siemens PRISMA). Patients were instructed to lie still with eyes open, which we confirmed with online eye-tracking. We used a multi-band echo planar imaging sequence (multi-band

Table 1 Clinical characteristics prior to scanning

Characteristic	Resistant tremor (n = 14)		Responsive tremor (n = 20)		Two-sample t-test / Fisher's exact test
Age	61.2 (4.3)		65.3 (2.1)		N.S.
Gender (male/female)	11/3		9/11		0.08
Disease duration (years)	2.3 (1.9)		5.2 (5.5)		N.S.
Hoehn and Yahr	2 (1–3)		2 (2–3)		N.S.
FAB	17.6 (0.5)		17.0 (1.1)		N.S.
MMSE	29.4 (0.8)		29.1 (1.5)		N.S.
LEDD	346.9 (207.5)		529.0 (63.9)		0.04
Levodopa usage	86%		95%		N.S.
Dopamine agonist usage	21%		30%		N.S.
Beta-blocker usage	28%		10%		N.S.
Levodopa challenge test (prior to scanning)	Resistant tremor (n = 14)		Responsive tremor (n = 20)		ANOVA (repeated measures)
	OFF	L-DOPA	OFF	L-DOPA	
MDS-UPDRS					
Total	40.0 (18.5)	28.1 (14.7)	46.5 (14.2)	21.4 (10.6)	$F(1,32) = 35.7; P < 0.001$
Limb bradykinesia	13.8 (7.6)	8.1 (5.7)	17.1 (6.0)	9.5 (5.4)	N.S.
Limb rigidity	4.2 (3.6)	2.3 (2.7)	5.3 (2.6)	2.3 (2.0)	N.S.
Limb resting tremor					
MA-UE	3.1 (0.7)	2.9 (0.7)	2.8 (0.7)	1.5 (1.1)	$F(1,32) = 22.2; P < 0.001$
MA-LE	1.4 (1.0)	1.4 (1.0)	1.7 (1.0)	0.1 (0.2)	$F(1,32) = 40.2; P < 0.001$
LA-UE	1.3 (1.3)	1.3 (1.3)	1.3 (1.2)	0.5 (1.1)	$F(1,32) = 15.2; P < 0.001$
LA-LE	0.8 (0.8)	1.6 (0.7)	1 (1.0)	0.2 (0.5)	$F(1,32) = 7.2; P = 0.01$
Lip	0.4 (0.6)	0.2 (0.4)	0.4 (0.6)	0.1 (0.2)	N.S.
Constancy	3.9 (0.3)	3.6 (0.6)	3.4 (1.0)	0.9 (0.5)	$F(1,32) = 46.2; P < 0.001$
Limb postural tremor					
MA	1.7 (0.7)	1.2	2.3 (1.1)	0.7 (0.7)	$F(1,32) = 10.2; P = 0.003$
LA	0.9 (0.9)	0.9	0.9 (1.1)	0.3 (0.6)	$F(1,32) = 4.3; P = 0.045$
Limb kinetic tremor					
MA	1.1 (0.8)	0.9	1.0 (0.9)	0.5 (0.6)	N.S.
LA	0.8 (0.7)	0.6	0.7 (0.7)	0.2 (0.4)	N.S.
Accelerometer tremor [log(power)]					
Rest (mean of 3×1 min)	6.6 (3.0)	5.1 (3.0)	5.3 (3.2)	0.04 (1.6)	$F(1,32) = 12.2; P = 0.001$
Cognitive stress (mean of 3×1 min)	9.7 (3.1)	8.9 (2.9)	7.8(3.4)	2.5 (2.2)	$F(1,32) = 19.9; P < 0.001$

Disease characteristics of all patients included for the functional MRI study are shown (Hoehn and Yahr: median, minimum and maximum scores in parentheses; other parameters: mean, standard deviation in parentheses). Disease severity of each patient was measured using the Hoehn and Yahr stages (maximum = 5) and the MDS-UPDRS part III (maximum score = 132). Limb rigidity was calculated as the sum of MDS-UPDRS item 3 (excluding item 'Neck'; min–max score 0–16), limb bradykinesia as the sum of items 4–8 (min–max score 0–40). All tremor (sub)items of the UPDRS are displayed (items 15–17; min–max score 0–4 per sub-item). Tremor is also objectified during the levodopa challenge test using accelerometer recordings of the most affected upper extremity. Accelerometer power values are log transformed and averaged across three trials of 1 min in rest and during cognitive stress (counting backwards from 100 to 7 as fast as possible). Group comparisons were carried out using two-sample t-tests (two-tailed) and a repeated measures ANOVA for quantitative variables and Fisher's exact test for categorical variables. FAB = Frontal Assessment Battery; LA = least affected; LE = lower extremity; LEDD = levodopa equivalent daily dose; MA = most affected; MMSE = Mini-Mental State Examination; N.S. = not significant.; UE = upper extremity.

acceleration factor = 4; repetition time = 0.859 s; echo time = 34 ms; 44 axial slices; voxel size = 2.2 mm isotropic; field of view = 225 mm; scanning time ~10 min; 700 images). The first five images were discarded. High resolution anatomical images were acquired using a magnetization-prepared rapid gradient-echo sequence (repetition time = 2.300 s; echo time = 3.03 ms; voxel size = 1.0 mm isotropic; 192 sagittal slices, field of view = 256 mm; scanning time ~5 min).

Functional MRI images were analysed using SPM12 (<http://www.fil.ion.ucl.ac.uk>) and FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). First, we used ICA-AROMA (independent component analysis-based automatic removal of motion artefacts) to remove noise components in an automated, observer-

independent manner (Pruim *et al.*, 2015). As ICA-AROMA is scripted in FSL, we performed several preprocessing steps in FSL: image registration, motion correction, non-brain removal, spatial smoothing (using a Gaussian kernel of 5 mm full-width at half-maximum) and grand-mean intensity normalization (Jenkinson and Smith, 2001). All components were visually checked. Next, output images from ICA-AROMA (realigned and in native space) were further preprocessed in SPM12: (i) co-registered to structural MRI image; (ii) normalized to MNI (Montreal Neurological Institute) space; and (iii) spatially smoothed using a 6 mm Gaussian kernel (resulting in a net smoothing kernel of 7.8 mm). Structural images were segmented and normalized using a unified segmentation approach (Ashburner and Friston, 2005).

Tremor-related EMG activity

We used the same procedures as previously (Dirkx et al., 2016, 2017). During MRI scanning, we measured activity of the most affected forearm muscles (extensor digitorum communis and flexor carpi radialis muscles) using MRI-compatible EMG (Brain Products; sampling frequency = 5000 Hz). Preprocessing included (i) removal of MRI-induced artefacts (van der Meer et al., 2010); and (ii) high-pass filtering >2 Hz to remove slow frequency drifts and rectification to capture the frequency of muscle bursts (using FieldTrip). Subsequently, we used FieldTrip to calculate time-frequency representations between 2–8 Hz in steps of 0.001 s using a 2 s Hanning taper, resulting in a 0.5 Hz spectral resolution. For each patient, we calculated the time course of the EMG at each subject's individual tremor frequency ± 1.5 Hz [dopamine-resistant; placebo: 4.2 ± 0.2 Hz, levodopa: 4.2 ± 0.1 Hz; dopamine responsive; placebo: 4.9 ± 0.2 Hz, levodopa: 4.8 ± 0.2 Hz, mean \pm standard error of the mean (SEM)]; Medication \times Group: [$F(1,32) = 0.64$, $P = 0.43$]. This resulted in patient-specific regressors describing fluctuations in tremor amplitude (EMG-amp). To remove outliers, data were logarithmically transformed and z-normalized within subjects. The first temporal derivative of the EMG-amp regressor was calculated to account for changes in tremor amplitude (EMG-change).

Tremor-related brain activity

In SPM12, we carried out a multiple regression analysis at the first level for each subject using a general linear model (GLM) including two regressors of interest (EMG-amp and EMG-change after haemodynamic convolution) and two regressors of no interest (average signal across the whole brain and bilateral ventricles to correct for non-neural noise) (Power et al., 2014). Parameter estimates for all regressors were obtained by maximum likelihood estimation.

First-level contrast images showing tremor-related activity (i.e. EMG-amp and EMG-change per session and averaged across sessions) as well as dopaminergic effects on tremor-related activity (EMG-amp and EMG-change in placebo versus levodopa) entered a second-level analysis (random effects). We used a one-sample *t*-test to test for effects across all 34 patients. To test for differences between patients with a dopamine-responsive versus dopamine-resistant tremor, we used a 2×2 full-factorial design with factors Group (between-subject) and Medication (within-subject). Specifically, we looked for main effects of group, medication and an interaction of both factors.

The analysis outlined above showed increased tremor-related activity in patients with dopamine-resistant versus dopamine-responsive tremor in a cerebellar region that included the deep cerebellar nuclei. As the contribution of dentate nucleus, interposed nucleus and fastigial nucleus to Parkinson's disease tremor differs (Muthuraman et al., 2018), we *post hoc* compared tremor amplitude-related activity between these nuclei using one-way ANOVA in the dopamine-resistant group. The deep cerebellar nuclei were defined using the Anatomy toolbox (based on Diedrichsen et al., 2011); average activity was calculated using MarsBar (Brett et al., 2002).

Regions of interest

We focused on brain regions consistently involved in Parkinson's tremor, i.e. the contralateral motor cortex (MC), [Brodmann area (BA) 4/6, 3712 mm³] (Helmich et al., 2011), contralateral ventrolateral thalamus (VLpv, 768 mm³) (Morel et al., 1997), ipsilateral cerebellum (lobule V/VI, 1416 mm³) (Helmich et al., 2011), and contralateral internal globus pallidus (GPi, 664 mm³) and external globus pallidus (GPe, 2256 mm³), from the Basal Ganglia Human Area Template toolbox (Prodoehl et al., 2008). Given our previous findings showing specific effects of dopaminergic medication on tremor-related activity in the GPi and VLpv (Dirkx et al., 2017), we focused the Group \times Medication interaction on these regions. Statistics were performed at the voxel level, and we corrected for multiple comparisons using the family-wise error (FWE) correction. Finally, we explored group differences in tremor-related activity outside these regions of interest, by performing a whole brain search with a cluster-forming threshold of $P = 0.001$, correcting for multiple comparisons at the whole brain using a threshold of $P < 0.05$ FWE corrected (Eklund et al., 2016). We used the SPM Anatomy Toolbox (Eickhoff et al., 2005) to locate detected clusters.

Physio-physiological interaction

The univariate analysis revealed several new group-specific clusters of tremor-related activity. We tested whether these regions showed tremor-specific functional connectivity with cerebral regions involved in tremor. For this, we performed a physio-physiological interaction analysis (PPI) (Friston et al., 1997) with the seed region's brain activity (blood-oxygen level-dependent, BOLD), fluctuations in tremor-amplitude (EMG), and their interaction (PPI, calculated as the unconvolved BOLD times unconvolved EMG signal with subsequent haemodynamic convolution) (Fig. 4). We also included the usual regressors of no interest (whole brain and lateral ventricle signal). Contrasts specifying the physio-physiological interaction entered a second level analysis. We used a two-sample *t*-test to look for group-specific interaction differences in any node of the tremor circuitry.

Supplementary analyses

We carried out five supplementary analyses to further specify our main findings. First, we scrutinized the criteria to define dopamine-resistant and responsive tremor, given that there is currently no consensus on this matter. Thus, we added a data-driven, automated two-step clustering approach based on clinical and electrophysiological tremor parameters to distinguish between patients with a dopamine-responsive versus a dopamine-resistant tremor (Dirkx et al., 2018). This procedure confirmed the presence of dopamine-resistant and dopamine-responsive subtypes in our sample, but the overlap with our clinically defined subgroups was not complete. Thus, we re-analysed the functional MRI data using the data-driven classification.

Second, we explored whether brain regions showing group-specific tremor-related brain activity (i.e. cerebellum and superior parietal cortex for dopamine-resistant tremor; OP4 for dopamine-responsive tremor) were functionally connected to

our previously identified tremor circuit, using seed-based analyses.

Third, we used dynamic causal modelling (DCM) to replicate our previous finding that dopamine reduces tremor-related activity by increasing the self-inhibitory connection of the VLpv, and that this effect is greater in dopamine-responsive patients (Dirkx *et al.*, 2017). As DCM requires that identical cerebral nodes are used in each model that enters a model comparison, we could not use DCM to test whether these group-specific regions had group-specific influences on the tremor circuit.

Fourth, given that dopamine inhibits thalamic tremor-related activity, we calculated the genetic expression of dopamine receptor D1 (DRD1) and D2 (DRD2) in the VLpv, using post-mortem data ($n = 6$) from the Allen Human Brain atlas (Hawrylycz *et al.*, 2015).

Fifth, we investigated whether differences in cerebellar activity between groups could be explained by an increased amount of tremor in the dopamine-resistant group during the levodopa session. For this, we *post hoc* compared mean beta values (extracted from the cerebellar region showing a whole-brain corrected main effect of Group) for each session separately using two-sample *t*-tests. Furthermore, we tested whether there was a between-session difference in tremor variance (measured using EMG) by calculating the coefficient of variation and performing a repeated measures ANOVA. In addition, we repeated our functional MRI analyses after adding two covariates describing tremor amplitude of the most affected upper extremity and tremor constancy (derived from the MDS-UPDRS item 17 measured on both scanning days) to our random-effects analysis. We also investigated whether usage of beta-blockers influenced cerebellar activity by repeating the functional MRI analyses while excluding two dopamine-resistant patients who used a beta-blocker with a possible incomplete wash-out during scanning sessions.

Data availability

The data are not publicly available due to their containing information that could compromise the privacy of research participants and written consent for data sharing was not obtained.

Results

Study population

Of 83 tremulous Parkinson's disease patients who underwent a levodopa challenge test, we selected 19 patients with a relatively dopamine-resistant tremor and 21 with a relatively dopamine-responsive tremor (Table 1). After scanning all 40 patients, we included 14 dopamine-resistant and 20 dopamine-responsive tremor patients in our analyses (Table 1 and Fig. 1B–D for clinical characteristics). We found a significant difference in dopamine response (Levodopa versus Placebo) between both groups for total MDS-UPDRS scores and for MDS-UPDRS limb resting tremor items (most-affected side, amplitude and constancy), but not MDS-UPDRS non-tremor items (limb bradykinesia

and rigidity). Importantly, both groups showed an overall response to dopaminergic medication (Table 1) confirming that the dopamine-resistance of tremor was not a consequence of general failure of levodopa effectiveness, e.g. because of gastrointestinal malabsorption.

Tremor-related brain activity

During the placebo session (when tremor was most pronounced), patients showed significant tremor-related activity within the cerebello-thalamo-cortical circuit (Fig. 2 and Table 2). This replicates previous findings. We did not find significant tremor-change related activity in the GPi or GPe (Helmich *et al.*, 2011; Dirkx *et al.*, 2016, 2017). There were no brain regions showing a Medication \times Group interaction, and there were no main effects of Medication on tremor-related activity. When testing for differences between groups (collapsed over medication sessions), we found that patients with a dopamine-resistant tremor had significantly more tremor amplitude-related activity than dopamine-responsive patients in the cerebellum—hereafter referred to as CBLM2: lobule IV (13%), lobule V (11%), vermis IX (11%) and deep cerebellar nuclei [nucleus fastigii (11%), interposed nucleus (4%)]; percentages correspond to fraction of cluster located within each anatomical region, ipsilateral to the side of tremor (Fig. 3A). Tremor-related activity tended to differ between the three deep cerebellar nuclei [$F(2) = 3.16$; $P = 0.053$], and was highest for the interposed nucleus (fastigial nucleus versus interposed nuclei) [$t(13) = 2.3$; $P = 0.04$], fastigial nucleus versus dentate [$t(13) = 2.7$; $P = 0.02$] and interposed versus dentate nuclei [$t(13) = 3.0$; $P = 0.01$] (Fig. 3A). This is in line with a recent study linking cerebellar tremor oscillations of Parkinson's disease to the interposed nucleus (Muthuraman *et al.*, 2018). Patients with dopamine-resistant tremor also had significantly more tremor change-related activity in the superior parietal cortex [SPC, area 7P (24%) and 7A (23%)] (Fig. 3) contralateral to the tremor, as compared to patients with dopamine-responsive tremor. Conversely, patients with a dopamine-responsive tremor had significantly more tremor amplitude-related activity in the VLpv and somatosensory regions of the parietal cortex [from now referred to as OP4: OP4 (31%), OP3 (8%), TE3 (10%) and Brodmann area 3b (7%)] contralateral to the tremor (Fig. 3B).

Physio-physiological interaction

Dopamine-responsive patients had significantly greater tremor-related functional connectivity between OP4 and the VLpv than dopamine-resistant patients [voxel coordinates: $(-16 \ -18 \ 2)$ mm (x, y, z); $T = 3.33$, $P = 0.024$ FWE-corrected]. This fits with our main finding that dopamine-responsive patients show greater tremor-related activity in both OP4 and VLpv and suggests that these regions interact with each other during tremor episodes (Fig. 4).

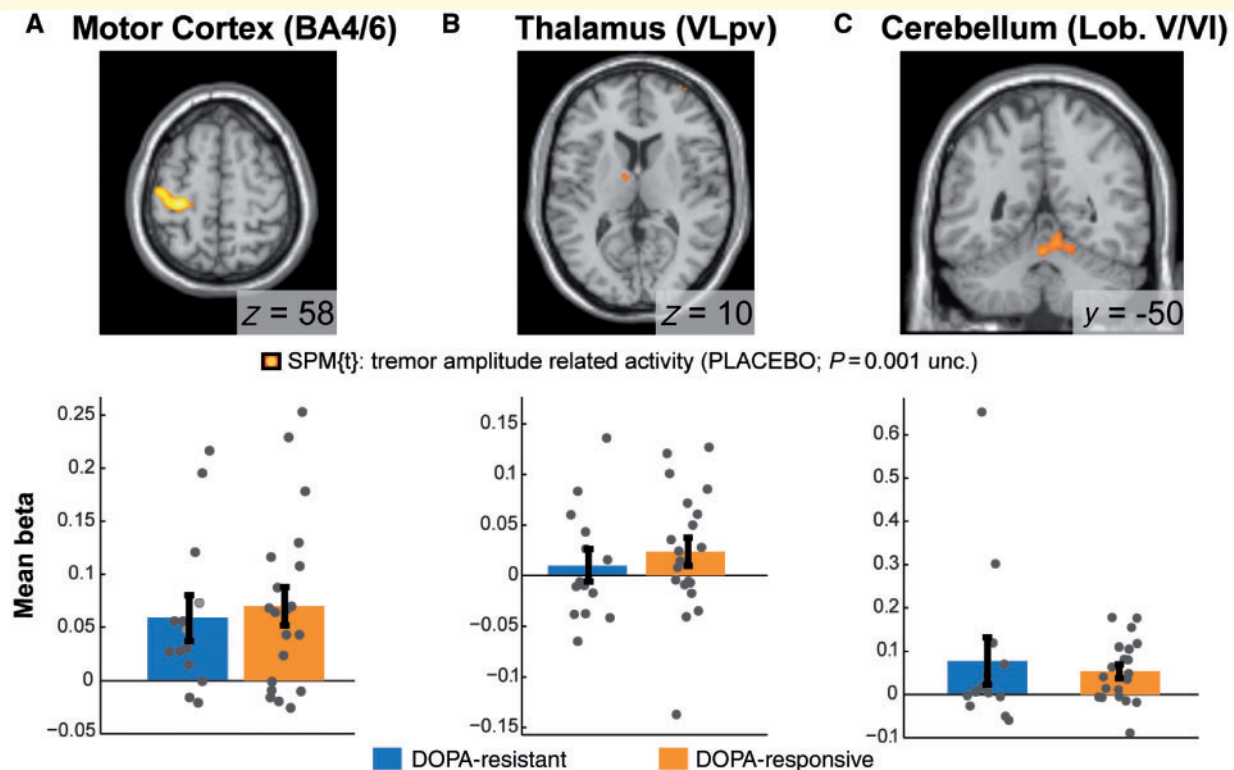


Figure 2 Similarities in tremor-related activity across both subgroups. Figure displaying tremor amplitude-related activity across both groups (top) and comparison of mean beta values between groups (bottom). Both subgroups show similar tremor amplitude-related activity in the cerebello-thalamo-cortical circuit. The bar graphs show the mean beta values (\pm SEM) and all individual datapoints within each region of interest. The left side of the figure depicts the left side of the brain (which was always contralateral to the side of the tremor). A threshold of $P = 0.001$ uncorrected was used for graphical purposes.

Conversely, there were no significant interactions for Resistant > Responsive contrasts.

Supplementary analyses

For details, see the online [Supplementary material](#). First, a data-driven automated clustering method using subjective (i.e. MDS-UPDRS) and objective (i.e. accelerometry) measurements of tremor dopamine-responsiveness revealed three groups: dopamine-responsive, dopamine-resistant and an intermediate category. Six of 20 patients originally defined as dopamine-responsive fell into the intermediate category, and one was classified as dopamine-resistant. Conversely, only 1 of 14 dopamine-resistant patients was classified as intermediate. Functional MRI analyses on these new groups of 13 dopamine-responsive and 14 dopamine-resistant patients yielded roughly the same results.

Second, the clusters in the somatosensory cortex (i.e. OP4) and cerebellum (CBLM2), but not in the SPC, showed significant functional connectivity with all regions of the tremor circuit (except CBLM2 < > MC).

Third, we replicated our previous finding that, across all 34 patients, a model where dopamine influenced tremor-related activity by acting on the inhibitory self-connection of the VLpv was more likely than effects of dopamine on any

other possible connection or a 'null' model where dopamine did not exert any effect (Dirkx et al., 2017). Moreover, the probability of this model was significantly larger in patients with a dopamine-responsive versus dopamine-resistant tremor. This replicates our previous finding that the modulatory effect of dopamine onto the VLpv is higher in patients with a relatively dopamine-responsive tremor (Dirkx et al., 2017).

Fourth, in six post-mortem brains from the Allan Human Brain Atlas, we found a high genetic expression of the dopamine D2 receptor (compared to the dopamine D1 receptor) in the VLpv, but not the motor cortex or cerebellum. This supports our current and past DCM finding that dopamine inhibits (tremor-related) activity in the VLpv (Dirkx et al., 2017).

Fifth, activity in CBLM2 was significantly different between groups for both the placebo and the levodopa sessions separately (Fig. 2), indicating that this result was not driven by increased amount of tremor in the dopamine-resistant group. Additionally, we ruled out group and session-specific differences in the variability of the EMG regressor (coefficient of variation). Moreover, adding covariates describing tremor amplitude and constancy for each individual patient did not alter our functional MRI results.

Table 2 Clinical characteristics on scan days

MDS-UPDRS evaluated on scan days	Resistant tremor (n = 14)		Responsive tremor (n = 20)		ANOVA (repeated measures)
	Placebo	L-DOPA	Placebo	L-DOPA	
Total	40.4 (18.5)	35.9 (19.4)	44.9 (3.3)	32.6 (12.2)	$F(1,32) = 6.4; P = 0.016$
Limb bradykinesia	14.7 (8.1)	11.8 (8.5)	17.2 (5.5)	12.8 (8.7)	N.S.
Limb rigidity	4.2 (4.7)	3.8 (3.1)	5.5 (2.6)	4.2 (2.9)	N.S.
Limb resting tremor					
MA-UE	3.0 (0.7)	3.1 (0.7)	2.6 (0.9)	2.1 (1.3)	$F(1,32) = 5.12; P = 0.031$
MA-LE	1.4 (0.9)	1.3 (1.0)	1.4 (1.2)	0.9 (0.9)	N.S.
LA-UE	1.4 (1.1)	1.3 (1.1)	1.2 (1.3)	1.1 (1.3)	N.S.
LA-LE	0.9 (0.8)	0.9 (0.9)	1.1 (0.9)	0.7 (0.9)	N.S.
Lip	0.2 (0.4)	0.2 (0.4)	0.2 (0.4)	0.2 (0.4)	N.S.
Constancy	3.6 (0.7)	3.8 (0.6)	3.1 (1.3)	1.8 (1.4)	$F(1,32) = 11.8; P = 0.002$
Limb postural tremor					
MA	1.7 (0.9)	1.4 (1.1)	1.1 (0.7)	0.9 (0.7)	N.S.
LA	0.9 (0.7)	0.6 (0.6)	0.7 (0.6)	0.4 (0.6)	N.S.
Limb kinetic tremor					
MA	1.1 (0.6)	1.1 (0.7)	1.1 (0.6)	0.8 (0.6)	N.S.
LA	0.9 (0.7)	0.6 (0.6)	0.8 (0.6)	0.7 (0.7)	N.S.
EMG: tremor presence, % and frequency (Hz)					
MA-UE	100% (4.2)	93% (4.0)	100% (4.9)	60% (4.6)	NA
LA-UE	64% (4.3)	29% (3.9)	65% (4.8)	20% (4.0)	NA
MA-LE	14% (4)	0% (NA)	30% (4.8)	10% (4.5)	NA

Disease severity of each patient was measured using the MDS-UPDRS part III (maximum score is 132). Limb rigidity is calculated as the sum of MDS-UPDRS item 3 (excluding item 'Neck'; min-max score 0–16), limb bradykinesia as the sum of items 4–8 (min-max score 0–40). All tremor (sub)items of the UPDRS are displayed (items 15–17; min-max score 0–4 per sub-item). Group comparisons are done using a repeated measures ANOVA. LA = least affected; LE = lower extremity; MA = most affected; N.S. = not significant; N.A. = not applicable; UE = upper extremity.

Finally, exclusion of two dopamine-resistant patients with a possible incomplete beta-blocker washout (due to long half-life) revealed the same significant CBLM2 cluster indicating that beta-blocker usage did not influence our results.

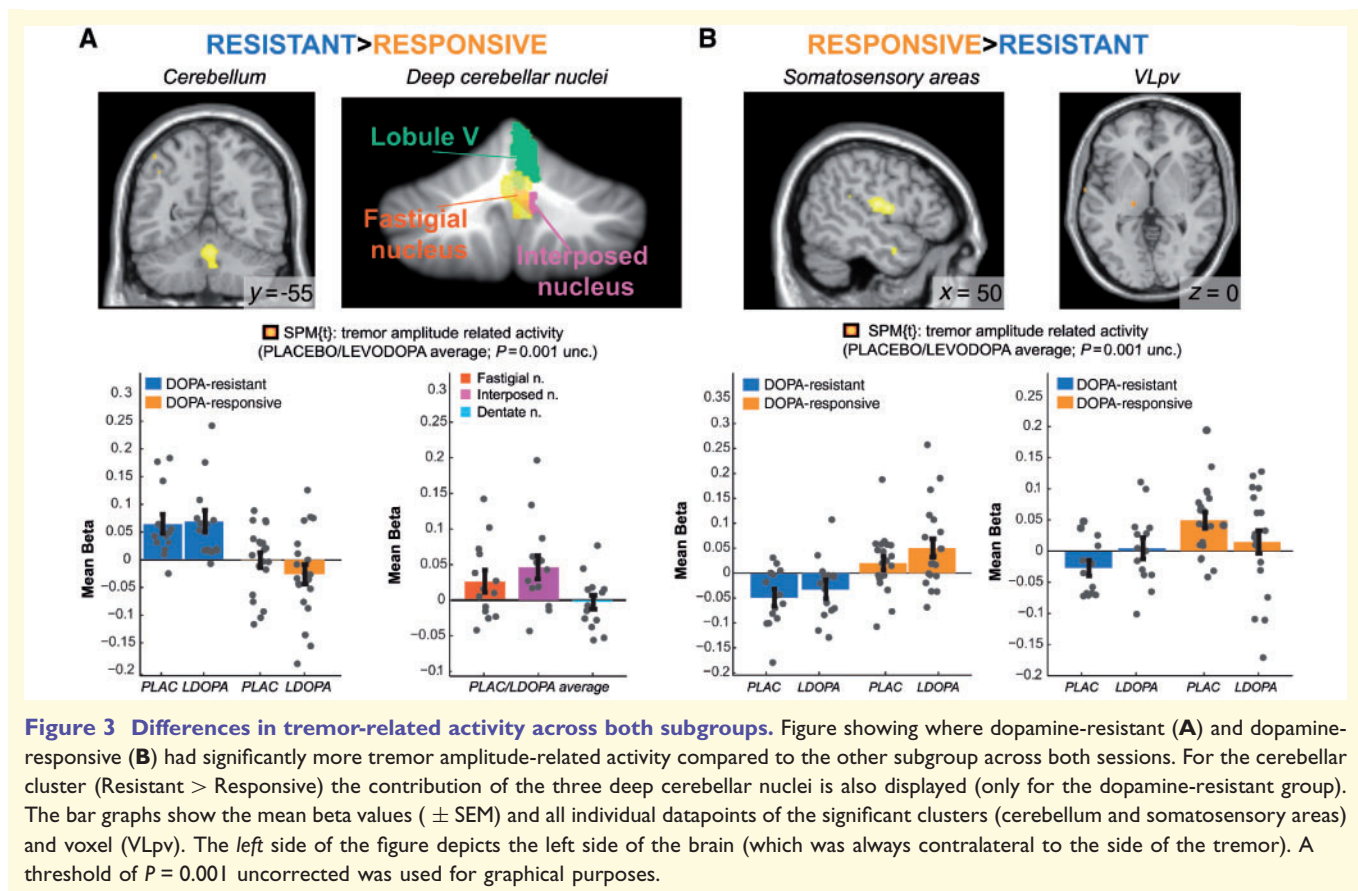
Discussion

We investigated the cerebral mechanisms underlying dopamine-resistant versus dopamine-responsive tremor in 34 Parkinson's disease patients. Informed by earlier work, we hypothesized that dopamine-resistant tremor is associated with increased tremor-related activity in non-dopaminergic areas of the tremor circuitry, and more specifically in the cerebellum. In both groups, tremor was associated with cerebral activity in a cerebello-thalamo-cortical circuit, replicating previous findings (Dirkx *et al.*, 2016). There are three main new findings. First, patients with dopamine-resistant tremor showed increased tremor amplitude-related activity in the cerebellum (lobules IV/V/IX and cerebellar nuclei, particularly the interposed nucleus). In these patients, those non-dopaminergic regions might be involved in the generation of dopamine-resistant tremor. Second, patients with dopamine-responsive tremor had increased tremor amplitude-related activity in the VLpv nucleus of the thalamus, in parietal somatosensory areas (OP4), as well as increased tremor-related connectivity between OP4 and VLpv. This suggests that tremor-related

somatosensory afference influences regions involved in tremor generation, which in turn may affect the dopamine-responsiveness of the tremor. Third, levodopa targets tremor-related activity in the VLpv of both groups (in line with Dirkx *et al.*, 2017)—but this effect is greater in dopamine-responsive patients (Supplementary material and Dirkx *et al.*, 2017). These three findings suggest that dopamine-resistance in Parkinson's disease tremor may be explained by increased excitatory cerebellar influences and reduced somatosensory influences onto the cerebellar thalamus (VLpv), making this key thalamic node of the tremor circuit resistant to dopaminergic inhibition.

The tremor circuitry of dopamine-responsive versus dopamine-resistant tremor

This work strongly builds on previous work where we found tremor amplitude-related activity in a cerebello-thalamo-cortical motor loop of Parkinson's disease patients (Helmich *et al.*, 2011; Dirkx *et al.*, 2016, 2017). Here, we replicate this result and show that this basic tremor circuit is present in both dopamine-responsive and dopamine-resistant tremor. We also found four brain regions where tremor-related activity differed between groups. As these effects were unrelated to levodopa, involvement of these regions in dopamine-responsive and resistant tremor



is not explained by (subthreshold) effects of dopamine in the resistant group. Instead, the differential involvement of these four regions may reflect altered efferent or afferent processing of tremor signals, which in turn could influence the resilience of tremor to dopaminergic intervention, as elaborated below.

Patients with a dopamine-resistant tremor showed increased tremor amplitude-related activity in the cerebellar cortex (lobules IV/V/IX) and in the deep cerebellar nuclei. This cerebellar cluster is located closely to the cerebellar region where both groups showed tremor-related activity in this and earlier studies (Helmich *et al.*, 2011; Dirkx *et al.*, 2016, 2017). Part of this region (lobules IV/V) has been associated with ipsilateral limb movement (Kelly and Strick, 2003; Ramnani, 2006). Lobule IX seems essential for visual guidance of movement (Glickstein *et al.*, 1994), although recent functional connectivity studies suggest it may also be part of a tertiary somatomotor representation (Yeo *et al.*, 2011). The dentate and interposed nuclei primarily control limb movement via cerebello-thalamo-cortical pathways, whereas the fastigial nucleus is involved in axial, proximal and ocular motor control (Diedrichsen and Bastian, 2014; Zhang *et al.*, 2016). The presence of tremor-related activity within the deep cerebellar nuclei, and specifically in the interposed nuclei (Fig. 3), is in line with recent EEG findings showing that the cerebellar source

of Parkinson's disease tremor is closest to the interposed nucleus (Muthuraman *et al.*, 2018), and that α -synuclein aggregation in the cerebellar deep nuclei of Parkinson's disease patients might be linked to the occurrence of (dopamine-resistant) tremor (Seidel *et al.*, 2017). There are at least three different mechanisms that could explain the involvement of the cerebellar nuclei in dopamine-resistant tremor, with different implications for dopaminergic intervention. First, given that the interposed nucleus sends glutamatergic projections to the VLpv (Diedrichsen and Bastian, 2014), patients with dopamine-resistant tremor may have increased thalamic excitation. This can explain why a dopaminergic intervention (which targets the VLpv) (Dirkx *et al.*, 2017) is insufficient to reduce tremor. In this case, the enhanced excitatory drive from the cerebellar nuclei to VLpv may be balanced by higher doses of dopaminergic medication. This would fit the theory that tremor suppression is a threshold phenomenon with respect to the dose of levodopa required (Obeso *et al.*, 2017). Second, given that there are multiple oscillators involved in Parkinson's disease tremor (Cagnan *et al.*, 2014), increased cerebellar activity may reflect oscillatory activity in the deep cerebellar nuclei. In this case, higher doses of dopaminergic medication would not overcome dopamine resistance, given that the oscillator in the thalamus is replaced by an oscillator in the deep cerebellar nuclei. This 'whack-a-mole'

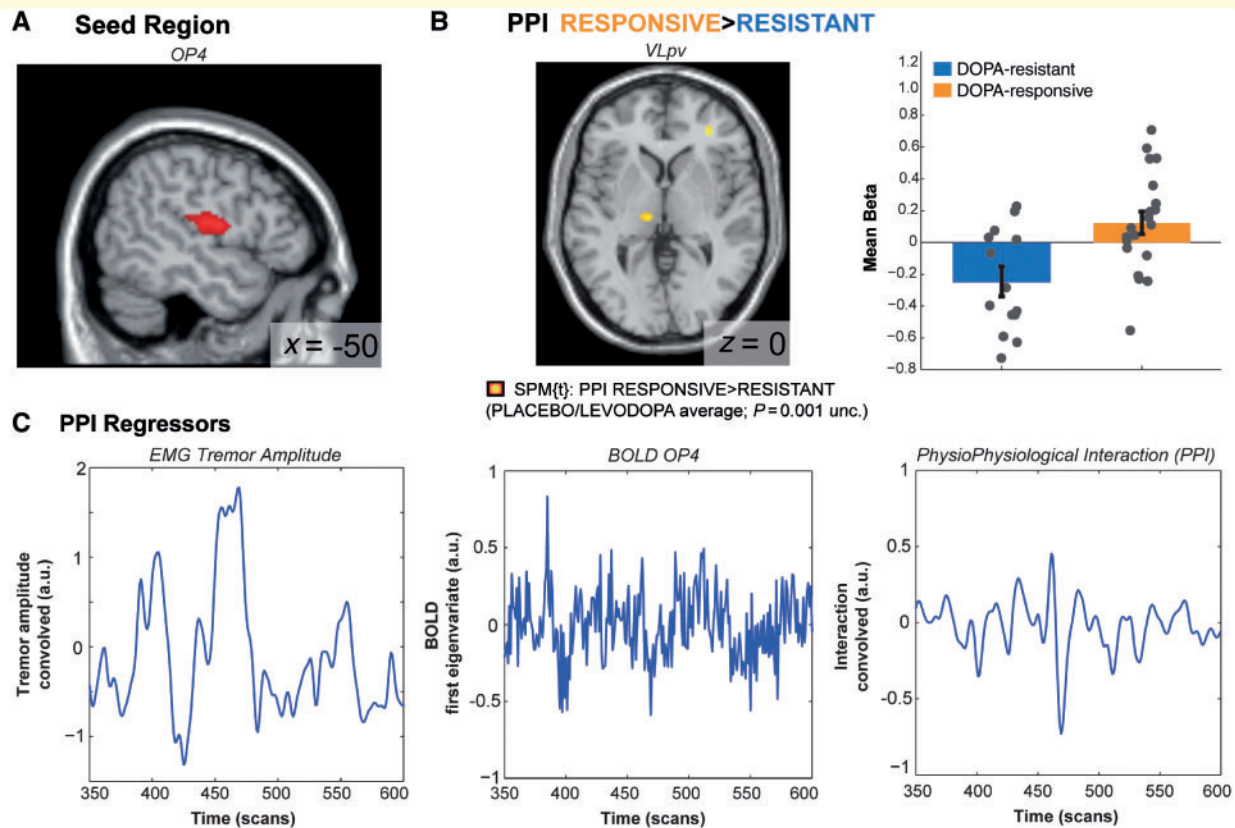


Figure 4 Physio-physiological interaction OP4 > VLpv. Illustration of tremor-related functional connectivity (physio-physiological interaction) between OP4 and VLpv for dopamine-responsive > dopamine-resistant patients. (A) Seed region (OP4). (B) Results of the PPI showing significant tremor-related functional connectivity between OP4 and VLpv for dopamine-responsive patients. A threshold of $P = 0.001$ uncorrected was used for graphical purposes. (C) Example from a representative subject showing the regressors that are used for PPI analysis including EMG tremor amplitude (left), first eigenvariate BOLD response of the OP4 (middle) and the interaction between both regressors (right; calculated using the deconvolved regressors of the left and middle panels with subsequent haemodynamic convolution).

effect has been described for essential tremor, where phase specific deep brain stimulation of the thalamus suppressed the dominant tremor frequency only to be replaced with a new oscillation at a lower frequency (Cagnan *et al.*, 2017). Third, tremor-related activity in the interposed nucleus (which receives somatosensory afferent input) (Elble *et al.*, 1984) might represent altered afferent processing of tremor-related signals in dopamine-resistant tremor.

Patients with dopamine-responsive tremor also showed increased tremor amplitude-related activity in the VLpv and a cluster consisting of mostly primary and secondary somatosensory cortex (i.e. OP4, OP3 and BA3b) in addition to a small portion of auditory cortex (i.e. TE3). Although the mean beta values displayed in Fig. 2B suggest that activity in the VLpv was dependent on levodopa (in line with our current and previous DCM results) (Dirkx *et al.*, 2017), this did not reach significance, possibly due to a limited sample size (see limitations below). BA3b, a portion of the primary somatosensory cortex (Kaas, 2004), has relatively focal receptive fields, whereas OP4 and OP3 are portions of the human parietal operculum (Eickhoff *et al.*, 2006) with bilateral receptive fields. Both regions

have already been linked to Parkinson's rest tremor in previous EMG-functional MRI and EMG-MEG studies (Timmermann *et al.*, 2003; Pollok *et al.*, 2009; Helmich *et al.*, 2011). Here, we add the finding that tremor-related functional connectivity between VLpv and OP4 is increased in dopamine-responsive tremor patients. Previous studies have shown that a significant portion of tremor cells in the ventral intermediate nucleus of the thalamus (VIM, which encompasses the VLpv) responds to somatosensory stimulation (Lenz *et al.*, 1994), and that somatosensory responses in the macaque's homologue of the VLpv (i.e. VPLo) are delayed with respect to cortical responses (Butler *et al.*, 1992). Accordingly, we interpret the increased tremor-related thalamo-cortical connectivity observed in the dopamine-responsive group as an indication that tremor-related signals from the somatosensory cortex might drive thalamic tremor cells, rather than the other way around.

Patients with dopamine-resistant tremor also showed a cluster of tremor change-related activity in the superior parietal cortex (BA7A and 7P). Previous studies have linked the posterior parietal cortex to processing afferent tremor

Table 3 Tremor-related activity

Voxel level inference (regions of interest)							
Contrast	Anatomical label	Anatomical location	Hemisphere (wrt tremor)	Tremor activity type	MNI (x y z)	T	P (FWE corr.)
Across all subjects (n = 34)	MC	BA4 (60%) BA6 (26%)	Contralateral	Tremor amplitude	±42 -22 50	6.0	<0.001
	VLpv	VLpv (Morel Atlas)	Contralateral	Tremor amplitude	±8 -6 8	3.4	0.027
	CBLM	Lobule V (39%) Lobule VI (49%)	Ipsilateral	Tremor amplitude	±18 -50 -18	3.4	0.023
RESP > RESIST	VLpv	VLpv (Morel Atlas)	Contralateral	Tremor amplitude	±20 -18 4	3.3	0.031
Cluster level inference (whole brain corrected)							
Contrast	Anatomical label	Anatomical location	Hemisphere (wrt tremor)	Tremor activity type	Cluster size (voxels)	Local peak (MNI x y z)	P (FWE corr.)
RESIST > RESP	CBLM	Lobule IV (13%) Lobule V (11%) Vermis IX (11%) Nucleus fastigii (11%) Interposed nucleus (4%)	Ipsilateral	Tremor amplitude	224	±2 -52 -20	0.02
	SPC	BA7P (24%) BA7A (23%)	Contralateral	Tremor change	250	±16 -74 48	0.006
RESP > RESIST	Somato-sensory areas	OP4 (31%) OP3 (8%) TE3 (10%) BA3b (7%)	Contralateral	Tremor amplitude	570	±66 -8 4	<0.001

Table showing the results of one-sample t-tests across the entire cohort in the placebo session and two-sample t-tests between patients with a dopamine-resistant (RESIST) and dopamine responsive (RESP) tremor across both sessions. Both statistical methods (at cluster and voxel level) are displayed. For the whole brain method statistical inference at the cluster level was used (threshold cluster searching $P = 0.001$ uncorrected). Percentages in parentheses behind each anatomical location correspond to percentage of tremor-related cluster that overlaps with the specified anatomical cluster. BA = Brodmann area; CBLM = cerebellum; MC = motor cortex; OP = operculum; SPC = superior parietal cortex; TE = temporal area; wrt = with respect to.

input (Timmermann *et al.*, 2003; Pollok *et al.*, 2009), but the cluster found in this study lacks clear connectivity to any region of the tremor circuitry (Supplementary material), questioning its functional relevance in dopamine-resistant tremor.

The role of (altered) afferent processing in Parkinson's tremor

The findings of this study suggest that differential tremor-related activity between groups can be linked to differences in somatosensory processing, raising the possibility that resilience to dopaminergic medication can be (partly) explained by altered processing of afferent, tremor-related activity. Previous studies have debated the role of somatosensory feedback in modulating Parkinson's disease tremor. On the one hand, several studies indicate that tremor amplitude can be reduced or stopped/reset by (i) surgically removing the dorsal roots (Pollock and Davis, 1930); (ii) fixing joints of the trembling limb by application of a cast (Burne, 1987); or (iii) electrical stimulation of the median nerve (Britton *et al.*, 1993). On the other hand, tremor frequency and phase is not (or only to a small extent) affected by these or other interventions such as (i) weighing of the tremulous limb (Deuschl *et al.*, 1996); or (ii) mechanical displacement at wrist joint (Lee and Stein,

1981). Thus, somatosensory input may have a role in modulating tremor amplitude, but not the occurrence of tremor *per se*. Accordingly, Volkmann *et al.* (1996) have suggested that Parkinson's tremor may be generated via an intrinsic basal ganglia-thalamo-cortical motor loop, while an extrinsic spino-cerebellar-thalamo-cortical feedback loop stabilizes the 3–6 Hz tremor oscillations. Increased extrinsic stabilization of tremor oscillations may explain resilience of tremor to any intervention, including a dopaminergic one. The current findings cannot distinguish between afferent and efferent activity, but they suggest to investigate whether manipulating the effect of an afferent intervention (such as median nerve stimulation) (Britton *et al.*, 1993) on tremor is correlated with the dopamine response.

Effects of levodopa onto the tremor circuitry

This work confirms that levodopa acts on the inhibitory self-connection of the VLpv, and that this effect is greater in dopamine-responsive tremor patients (Supplementary material and Dirkx *et al.*, 2017). This dopaminergic effect onto a cerebellar receiving nucleus of the thalamus is physiologically plausible and likely inhibitory. First, the thalamus, including the VLpv, is known to receive dopaminergic projections from the mesencephalon (Brown *et al.*,

1979; Sanchez-Gonzalez *et al.*, 2005). Second, the Allen Human Brain Atlas indicates relatively high genetic expression of dopamine D2 (and not D1) receptors in the VLPv, which in the basal ganglia is associated with inhibitory effects (Albin *et al.*, 1989).

The fact that levodopa induced thalamic inhibition in both dopamine-responsive and resistant tremor patients may appear surprising, as the dopamine-resistant group showed no or little clinical effect of dopamine on tremor. This raises the possibility that dopamine altered thalamic processing to an extent that is insufficient (sub-threshold) to induce clinical effects. The observation that deep brain stimulation of the VIM, which exerts a more powerful (suprathreshold) inhibitory effect on local thalamic neurons, reduces Parkinson's disease tremor regardless of its dopamine response (Lozano *et al.*, 2002), would fit this explanation. The fact that dopamine reduces tremor primarily by targeting the VIM (Dirkx *et al.*, 2017) also explains why DBS of the subthalamic nucleus is another effective method of treating both dopamine-resistant and dopamine-responsive tremor. To rule out trivial explanations for a subthreshold effect, both groups were matched in terms of the dopamine responsiveness of other (non-tremor) symptoms. Nevertheless, it is possible that patients with a dopamine-resistant tremor need higher doses of levodopa to reach a threshold of sufficient thalamic inhibition. Although we cannot rule this out, both groups received a substantially higher dose during this experiment than their own morning dose (dopamine-responsive tremor: $54 \pm 9.7\%$; dopamine-resistant tremor: $71 \pm 7.5\%$; average \pm SEM, no group difference). This makes it unlikely that (dopamine-resistant) patients were under-dosed.

Effects of other neurotransmitters on Parkinson's tremor

The group-specific pattern of tremor-related brain activity in patients with dopamine-resistant and dopamine-responsive tremor was unaffected by levodopa. This raises the question whether other neurotransmitter systems may be responsible for dopamine-resistant tremor. Recent studies indeed suggest that Parkinson's tremor may also have a noradrenergic and/or serotonergic basis, and that this may (in part) explain dopamine-resistance (Doder *et al.*, 2003). For instance, data from the Parkinson's Progression Markers Initiative (PPMI) cohort show decreased raphe serotonin transporter availability in Parkinson's disease, and a correlation between serotonin transporter depletion and tremor severity (Qamhawi *et al.*, 2015; Pasquini *et al.*, 2018). Moreover, in these studies the raphe/putamen binding ratio of ^{123}I -FP-CIT correlated with the clinical dopamine response of tremor. This suggests that the serotonergic system may play a relatively larger role in patients with a relatively dopamine-resistant tremor. There is also evidence for a role of the

noradrenergic system in the generation of Parkinson's tremor (Isaias *et al.*, 2011), for example illustrated by the observation that Parkinson's tremor increases during cognitive stress (Zach *et al.*, 2017). Accordingly, tremor-dominant Parkinson's disease patients have less degeneration of the locus coeruleus (the main source of cerebral noradrenalin), and tremor increases after intravenous injection of adrenalin (Barcroft *et al.*, 1952). Whether or not the noradrenergic system plays a larger role in Parkinson's disease patients with a relatively dopamine-resistant tremor remains to be investigated. Future studies may focus on the exact contribution of (nor)adrenaline and serotonin on the generation of tremor, to further pave the road for new treatment strategies.

Interpretational issues

Because of our strict inclusion criteria, we could only include 14 dopamine-resistant patients for final analyses, raising the question whether this yielded sufficient power. On the one hand, we found robust tremor-related activity in the cerebello-thalamo-cortical circuit replicating previous studies (Helmich *et al.*, 2011; Dirkx *et al.*, 2016, 2017), suggesting that we had enough power to detect tremor-related activity. On the other hand, there was no significant Medication \times Group effect for tremor amplitude-related activity in the VLPv, suggesting that this study was underpowered for a univariate interaction effect. However, our connectivity analyses (which are more sensitive than simple univariate methods) (Dirkx *et al.*, 2016) were able to show a significant interaction effect on the VLPv self-connection, replicating previous results (Dirkx *et al.*, 2017). We investigated differences in cerebral activity between dopamine-resistant and dopamine-responsive patients, although it has yet to be established that there are indeed two phenotypes or if this rather reflects two ends of a normal spectrum. The results from our data-driven automated clustering approach (Supplementary material) support this idea, but it remains to be confirmed in prospective research.

Patients that were included into our functional MRI study were carefully selected on the basis of a levodopa challenge that was not placebo controlled (in a larger sample of 83 patients). It is well-known that Parkinson's disease patients are susceptible to the placebo response (Quattrone *et al.*, 2018). Indeed, the levodopa effect on Parkinson's disease symptoms was larger during the uncontrolled levodopa challenge (Table 1) than during the placebo-controlled MRI measurements (Table 2). Hence, it is possible that there were more dopamine-resistant patients than the 19 subjects included in our functional MRI study. However, the purpose of the present study was not to give a reliable estimate on the prevalence of dopamine-resistant patients, but to identify the cerebral differences between dopamine-responsive and dopamine-resistant patients. During scanning, we corrected for placebo effects by using a double-blind design where patients either received a placebo or levodopa-benserazide (200/50 mg). Thus, we

are confident that functional MRI differences between the two sessions cannot be explained by a placebo effect.

Conclusion

The findings suggest that interindividual clinical differences in the dopamine response of Parkinson's disease rest tremor can be explained by differences in tremor pathophysiology. While the cerebello-thalamo-cortical tremor circuit was involved in both dopamine-responsive and dopamine-resistant groups, patients with dopamine-resistant tremor had increased tremor-related activity in the cerebellar output nuclei and reduced cortical somatosensory influences on the ventrolateral thalamus, which in turn was less sensitive to dopamine. Together, these findings suggest that afferent and cerebellar influences on the ventrolateral thalamus determine the susceptibility of the VLpv to dopaminergic medication. These findings may have therapeutic implications, suggesting that an alteration of cerebellar reactivity and/or tremor-related processing may improve the clinical dopamine response of tremor.

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Competing interests

M.D., A.v.N., H.Z. and I.T. declare no competing interest. R.H. serves on the clinical advisory board of Cadent Therapeutics, and received honoraria from AbbVie. B.B. has received honoraria from serving on the scientific advisory board for Abbvie, Biogen, UCB and Walk with Path, has received fees for speaking at conferences from AbbVie, Zambon, Roche, GE Healthcare and Bial.

Supplementary material

Supplementary material is available at *Brain* online.

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